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Methodologies for the airbrush application of MALDI matrices

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Keywords:

Matrix-assisted laser desorption, matrix, airbrush, colloidal graphite, traditional matrices

Dedication:

Dedicated to the memory of Professor Peter J. Derrick, who encouraged me to undertake a career in research in mass spectrometry and originally introduced me to MALDI-MS. (Paul Gates 2017).

Abstract

There is still a need to develop reliable and robust matrix deposition methods for matrix-assisted laser desorption/ionisation mass spectrometry that are applicable to a range of matrices, solvents and analyte types. This paper presents a robust methodology for the airbrush application of matrices along with the implications of varying the set-up and airbrush parameters. A small number of organic analytes and metal salts are analysed in both positive and negative ion modes to exemplify this methodology. In the analyses with the airbrush deposited matrices, performance was enhanced when compared to standard pipette deposition with the need for a search for sweat spots greatly diminished due to the increase homogeneity of the matrix surface and resultant analyte spots. As expected, the graphite matrices were shown to specifically outperformed the organic matrices in negative ion mode.

Introduction

Although matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry (MS) is a well-established technique, there are still a range of issues regarding sample preparation and deposition onto the target plate. These result in the quality of spectra being, in many cases, dependent on user experience. It would be desirable to remove this dependence, resulting in spectral quality being more related to sample quality with a limited impact due to the analyst. There have been a several studies that have attempted to reduce this dependence through introducing a level of automation to the process by developing an airbrush deposition methodology.[1-4] An additional advantage of airbrush deposition, is that the matrix can be easily deposited over a larger area leading to a more homogenous coating than would be possible with traditional pipette based methods – this is very desirable in (for example) MALDI imaging studies.

The airbrush application of a MALDI matrix was studied in 2007 and 2008 as an alternative to spray-can deposition of colloidal graphite for the MALDI analysis of small biomolecules from a range of fruit samples,[5] rat brain tissue[6] and plant metabolites.[7] Another 2007 paper, detailed the application of 2,6-dihydroxy acetophenone by airbrush for the analysis of phospholipids,[8] whilst a third paper was published in the same year utilizing a vertically positioned airbrush to apply 2,5-dihydroxybenzoic acid to biological samples for imaging.[2] This last paper was the first to indicate any level of automation in the application of an airbrushed matrix – with the gas pressure trigger being permanently depressed and the plate moved underneath the flow. It also compared airbrush application with inkjet and electrospray deposition; finding the quality of matrix deposition comparable with that of inkjet printing, but that the reproducibility of airbrushing was lower due to its reliance on the operator's movements of the plate. In a study dealing with the airbrush application of MALDI matrices offering comparisons with other methods of matrix application – in this case thin layer chromatography (TLC) sprayers and a nebulizer - airbrushing was found to be superior, and the tendency for wetting, which had been noted by previous operators, was negated.[4] Over wetting can be detrimental to the analysis because of analyte blurring and loss of spatial resolution. Reducing the amount of solvent being deposited is essential to avoid this.[9] These previous studies formed the basis for this systematic study, with the aim to enhance the use of an airbrush, as well as reducing operational error and variability to the lowest possible level.

Experimental

Instrumentation

Airbrush deposition of the matrix was achieved using an Aztek/Testors A470 (Rockford, Illinois, USA) airbrush in the single action mode. A Revell 'Master Class' airbrush compressor (Elk Grove Village, Illinois, USA) was used to provide a pressure of 35 psi. A needle size of 0.7 mm was found to give the best results. The distance between the sample plate and the airbrush was fixed at 30 cm. The angle between the plate and the airbrush was fixed at 15 degrees, and the angle between the plate and the surface was fixed at 10 degrees. Five individual presses of the airbrush trigger were used to deposit the matrix. Matrices were applied over pre-deposited and dried analyte sample spots.

All mass analyses were performed on a 4700 Proteomics Analyzer (Applied Biosystems, Warrington, UK). This is a reflectron Time-of-Flight/Time-of-Flight (TOF/TOF) mass analyzer, used in reflectron mode. All spectra were recorded at 1000 shots per spectrum (comprised of 8 sub-spectra, over a 5 s run). The 200 Hz Nd/YAG laser was employed at a wavelength of 355 nm. The laser beam diameter is 50 μm with pulse energy of 12 μJ and pulse length of less than 500 ps. Data collection was fully randomised and features or trends which appeared in the majority of the resulting spectra where then reported.

Chemicals and matrices

The organic matrices used were: Sinapinic acid (SA), α-Cyano-4-hydroxycinnamic acid (CHCA), (Sigma-Aldrich, Gillingham, U.K.) and 2,5 – Dihydroxybenzoic acid (DHB) (Fisher Scientific, Loughborough, U.K.). The non-organic matrices used were Edelgraphit Graphite Aerosol Spray and Micronised Graphite (Graphite Trading Company, Halesowen, U.K.). All solvents used throughout the study were HPLC gradient grade (Fisher Scientific, Loughborough, U.K.).

The organic matrices were prepared to a concentration of 5 mg/mL in methanol. The colloidal graphite matrix was prepared by spraying the can into a vial until approximately 1 mL of fluid collected at the bottom. The solution was then made up by adding 7 mL of water. The micronised graphite matrix was made up by adding a volume of powder to a vial, which was tapped to ensure the powder settled and a flat surface was obtained. Twice this volume of water was added. Both graphite matrices were thoroughly shaken before each use.

Analytes studied were: rubidium, strontium and cobalt ICP standard nitrate salts (Ultra Scientific, N. Kingstown, Rhode Island, USA) and naringenin and roxithromycin (Sigma-Aldrich, Gillingham, UK). All analytes were made up to a concentration of 125 ppm in water (for the ICP standards) and 50/50 methanol/water (for the organic analytes).

Methodology

Airbrush Settings

Although double-action spraying allows finer control, as the user can control both the rate of flow of air through the brush and the quantity of matrix added, it is less reproducible as the exact proportions of the mix is dependent on two variables. Single-action spraying releases a set quantity of matrix into an uninhibited flow of air, theoretically giving identical mixes each time and thus a higher reproducibility. The finest airbrush needle available (0.30 mm) was found to 'clog' readily during use. Moving to broader airbrush needles (0.40 mm, 0.50 mm and 0.70 mm) reduces the amount of clogging. A 0.70 mm needle reduced clogging to a point where it is no longer a significant issue.

Compressor Settings

The compressor used for the experiments operates from a filled tank (maximum pressure 60 psi) that is then refilled when the internal pressure drops to approximately 20 psi. Although the compressor gives the option of spraying at maximum pressure, the pressure will begin to drop as soon as spraying has begun – immediately reducing the output pressure and affecting the reproducibility of the experiment. It was found that the most 'effective' output pressure was approximately 35 psi – where by a prolonged period of spraying (20 seconds) can be achieved at approximately the same pressure, such that the tank did not need to be refilled during the spraying of multiple plates. It should be noted that the actual output pressure varies during spraying, but this variation appears to be of the order of ±2 psi, and as such should not have a significant impact on reproducibility. This is significantly higher pressure than that used by previous workers, who utilized pressures of around 20 psi.[2,5-7]

Positioning of plate and airbrush

It was found that slightly canting the plate (by approximately ten degrees to the horizontal) improved the application quality. However, to achieve an effective coverage, the airbrush needed to be lowered by 15 degrees below horizontal. The spray intercepts the plate at a smaller angle in this arrangement, which appeared to lead to a more even distribution of droplets. The airbrush was mounted on a retort stand 30 cm (nozzle to front of plate) from the canted target. The distance is such that the spray plume

expands to an extent that it completely covers the target plate's surface without the need to move the airbrush. Previous studies have used nozzle-plate distances of between 8 cm and 25 cm,[2,4-8] necessitating the movement of the plate to ensure an even coverage, and as a result introducing additional variability, therefore counteracting the benefits of using the airbrush. Fig. 1 shows a photograph of the arrangement of airbrush and sample plate.

Insert figure 1 here.

Solvent Effects

Solvent usage affects the airbrush deposition in two distinct ways – it can affect the operation of the airbrush, as well as deposition of the matrix on the sample plate. Water was found to be the best liquid phase for both colloidal and micronised graphite, with regards to the physical process of spraying. Less clogging was found to occur with water, whilst methanol, dichloromethane and tetrahydrofuran led to increased clogging. Methanol was found to be the most effective solvent for organic matrices, with no clogging issues being noted. The solvent used has an effect on the quality of the applied surface, especially when coupled to the airbrush nozzle size. Using water as a solvent led to the formation of discrete droplets, especially for wider nozzles, whereas methanol tended to 'wet' the surface of the sample plate and form a more film like layer. Due to the rapid drying of methanol, and the transparent nature of the dried organic matrices, further studies are required to confirm the homogeneity of the matrix surface.

Spray Duration and the Effect of Graphite Thickness

The duration of spray utilized by Zhang *et al.* in 2007 (30-40 s) was initially tested,[5,6] but was found to lead to the formation of large droplets of matrix on the plate surface, which reduced the quality of the data retrieved. In the worst cases, these droplets began to move down the slope of the plate, leaving blurred trails – resulting in an inhomogeneous distribution of matrix. The other studies either failed to describe the spray period[2,4] or sprayed for longer (2 minutes).[8]

Through a process of trial-and-error, it was determined that constant application leads to the formation of sizable droplets on the sample plate. An alternative method for controlling the duration of the spray, and thus the quantity of matrix deposition, is by utilizing a number of discrete 'presses' (whereby the airbrush trigger is full depressed, and then released as rapidly as possible, to give a 'pulse' of air through the airbrush). It has been noted that the droplets on the plate formed in this manner are smaller, and thus will result in a more homogeneous surface for matrix action.

An investigation into the most effective number of presses for matrix performance was conducted with the graphite matrix. The primary finding was that there does not appear to be any general trend with respect to the behaviour of the overall signal intensity with the quantity of matrix deposited. However, there were some cases where the thickness of matrix affected the species detected, with a 'critical concentration' being required before meaningful results were recorded. Other spectra showed peaks starting to be lost from the spectrum at higher thicknesses of matrix. Thus, a compromise covering was required for general use, which typically occurred at around 5 or 6 'presses' resulting in significantly less matrix being deposited than with previous studies.[5-8]

The number of presses was found to have no effect on the resolution of any of the signals recorded, in either positive or negative ion mode. There also appears to be no trend with regards to the signal-to-noise ratios or intensity. Values recorded showed some variation in terms of both range and mean, but this no worse than would be expected with MALDI using traditional matrices and deposition methods. As a result, small changes in the thickness of matrix applied, seems to have little effect on the spectra quality. The implication of this is that airbrushing matrices is a fairly robust mechanism, with low sensitivity to the thickness of the deposited matrix layer. Thus, small variations in the described parameters (i.e. deposition period, solution concentration, airbrush output pressure and distance between the airbrush and plate) should not have a significant effect on the general analytical performance of the technique.

Insert figure 2 here.

Matrix homogeneity

The homogeneity of airbrush-applied matrices was probed through small area imaging of approximately 6 mm^2 sections of the target plate. Using negative mode, the most intense graphite peak (C_6^-), was studied for both micronised and colloidal graphite. See Fig. 2 for example images. As can be seen there is no preferential citing of the matrix resulting in a fairly even distribution. The points of higher intensity (blues and greens) correlate to regions of higher matrix concentration. In the case of micronised graphite, these regions are thought to be caused by coagulation of graphite particles. A previous study[1] has shown that traditional matrix deposition methods for organic matrices can often lead to the formation of 'rings' of matrix – where the matrix has preferentially crystallized on the edge of the engraved sample spots. This is highly undesirable and prevents automated acquisition of spectra. Airbrushing of matrices completely eliminates this issue.

Results and Discussion

Insert figure 3 here.

The performance of all matrices was studied with all analytes by both the deposition method and airbrush application method. As would be expected, all matrices gave similar performance in the positive ion analysis of the organic analytes by the deposition method. For example, Fig. 3 shows the results for the analysis of the antibiotic roxithromycin. All methods yielded identifiable intact molecular species, although colloidal graphite results in an abundant $[M+K]^+$ peak and improved signal-to-noise ratios, whereas the organic matrices (exemplified here by CHCA) result in an abundant $[M+Na]^+$ peak. This is in accordance with what has previously been observed when comparing graphite matrices to organic matrices.[10-12] In negative ion mode analysis, the organic matrices generally performed very poorly (as expected) often with no analyte related species observed. Spectra were almost totally dominated by the deprotonated ions of the matrices themselves, with little signal resulting from the analytes. However, the graphite matrices performed much better, yielding clean spectra containing $[M-H]^-$ and $M^{-\cdot}$ for the flavonoid naringenin (see Fig. 4 a and b).

Insert figure 4 here.

The performance of the organic matrices in the analysis of the metal nitrates was incoherent. In the positive ion mode, CHCA returned peaks for Rb^+ and $[Rb_2NO_3]^+$ in the analysis of rubidium nitrate, whereas a selection of mixed nitrate/nitrite cluster ions was observed in the analysis of strontium nitrate. In negative ion mode, as above, the organic matrices failed to yield any identifiable analyte peaks.

The airbrush applied organic matrices gave very similar results to those obtained with the deposition method. The airbrush applied graphite matrices had far superior performance, with positive ion analysis showing a mix of both positively charged metal ions and other metal related species and negative ion mode showing counterion adducts for all the analytes (see Fig. 4). Additionally, airbrushing matrices tends to lead to spectra slightly enhanced signal-to-noise ratios and a reduction in background noise.

Conclusions

A robust airbrushing method is presented here. The methodology is simple to use and set-up and shows a low susceptibility to user variation or differences in the thickness of the deposited matrix. However, the effectiveness of a matrix is not just dependant on the number of analytes it can successfully return analytically useful data for. The quality of the returned spectra is also significant. Spectra obtained from the organic matrices tend to have significant signals at lower *m/z* values due to matrix cluster ions which can hinder the identification of unknown analytes. The graphite matrices are demonstrated to outperform the organic matrices in all the analyses performed, especially in negative ion mode. For all analytes, there is minimal matrix associated peaks and other unidentified 'noise' present in the spectra, making analyte identification straightforward. Another significant benefit of air-brush application of matrices is that the 'coffee-ring' affect due to preferential crystallisation of the matrix around the edges of the sample spots is eliminated. This results in spectra with enhanced signal-to-noise ratios and enables automated analysis.

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Figure Captions:

Figure 1: Photograph showing the arrangement of the matrix spraying apparatus. The airbrush is held approximately 15 degrees below horizontal, whilst the plate is at an angle of approximately 10 degrees to the horizontal.

Figure 2: Example small area negative ion MALDI images of (a) airbrushed micronised graphite and (b) airbrushed colloidal graphite. Data represents an intensity display for *m/z* 72 (C_6^-).

Figure 3: Positive ion MALDI spectra for roxithromycin (Mw 836 Da), utilising a range of matrices: (a) deposited CHCA matrix; (b) deposited colloidal graphite; (c) airbrushed colloidal graphite. Base peaks absolute intensities are as follows: (a) *m/z* 158 at 8600 counts, (b) *m/z* 113 at 1200 counts and (c) *m/z* 875 at 6700 counts.

Figure 4: Sample spectra obtained using airbrushed graphite matrices. Spectrum (a) Naringenin positive ion; (b) Naringenin negative ion; (c) Rubidium nitrate positive ion; (d) Rubidium nitrate negative ion. Base peaks absolute intensities are as follows: (a) *m/z* 295 at 1765 counts, (b) *m/z* 271 at 2100 counts, (c) *m/z* 85 at 9200 counts and (d) *m/z* 62 at 4400 counts.

Figure 1:

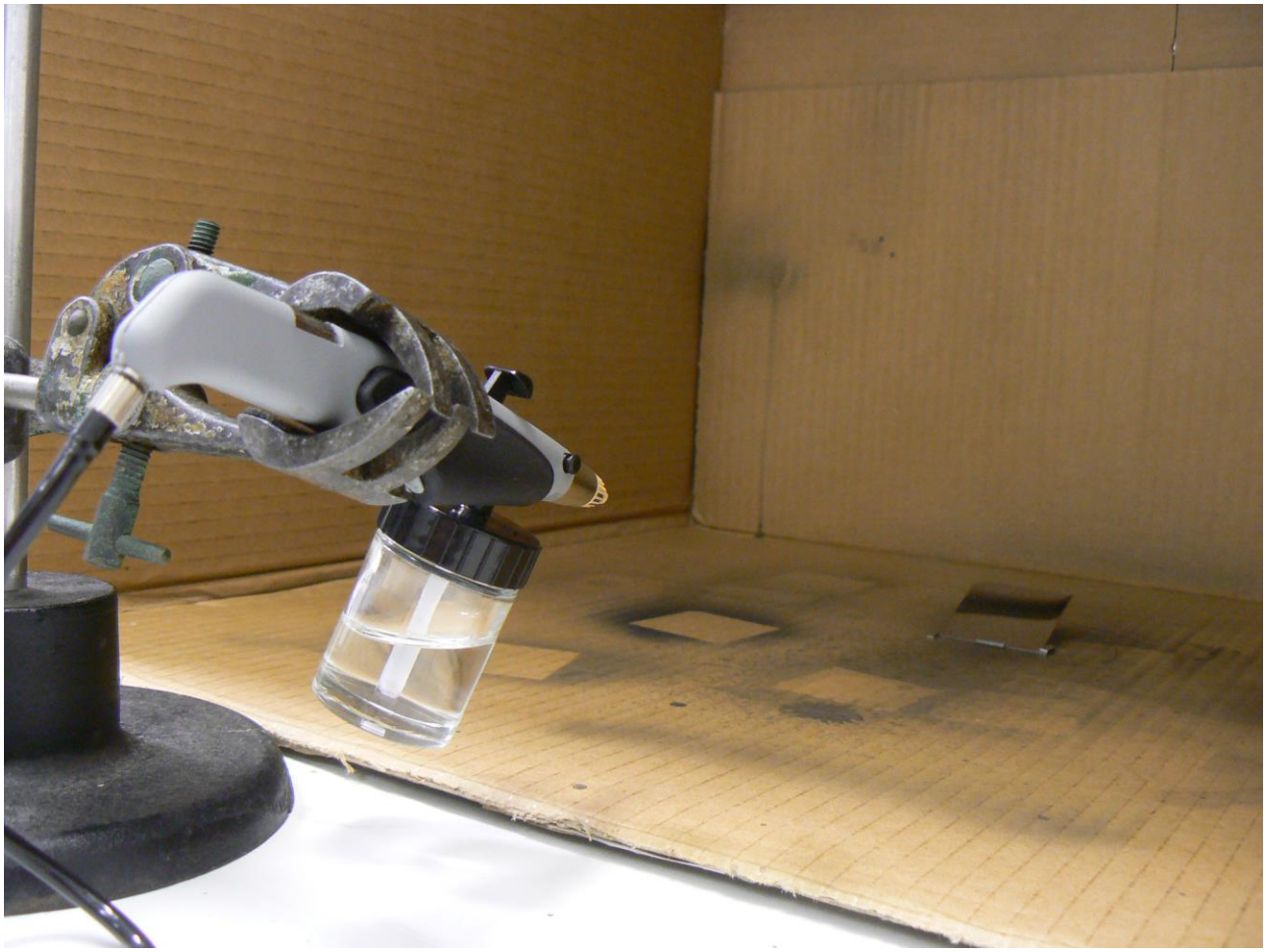


Figure 2:

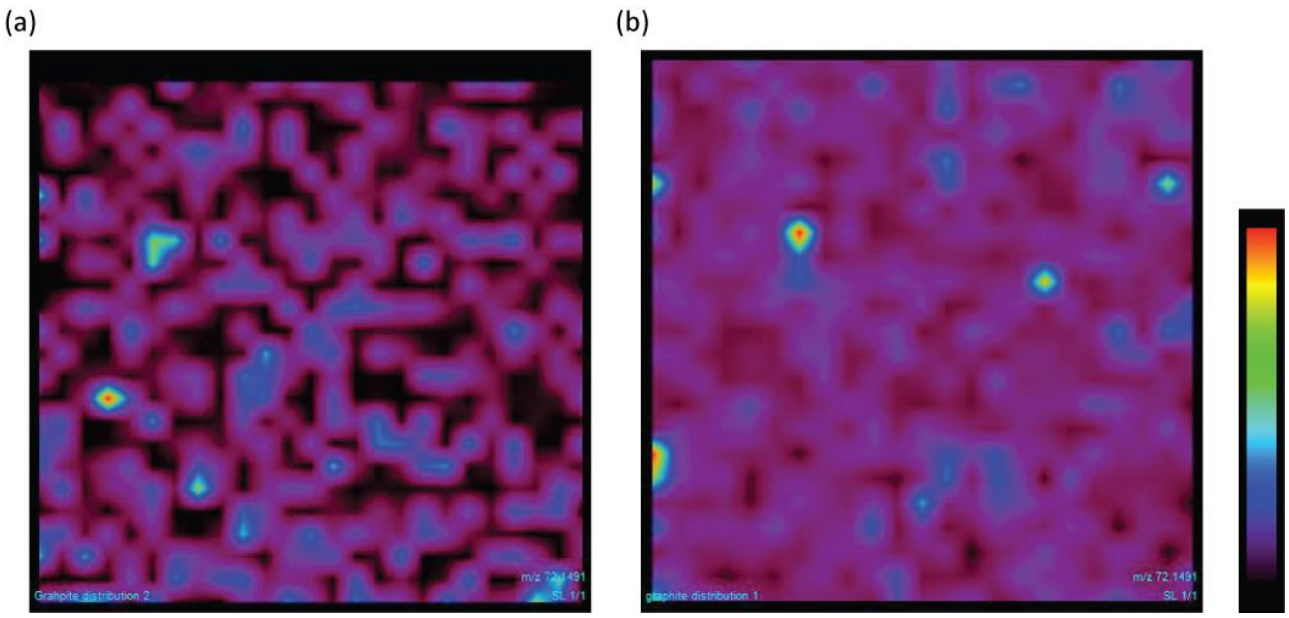

(a)
(b)
m/z 72.1491
Grahpite distribution 2
SL 1/1
m/z 72.1491
graphite distribution 1
SL 1/1

Figure 3:

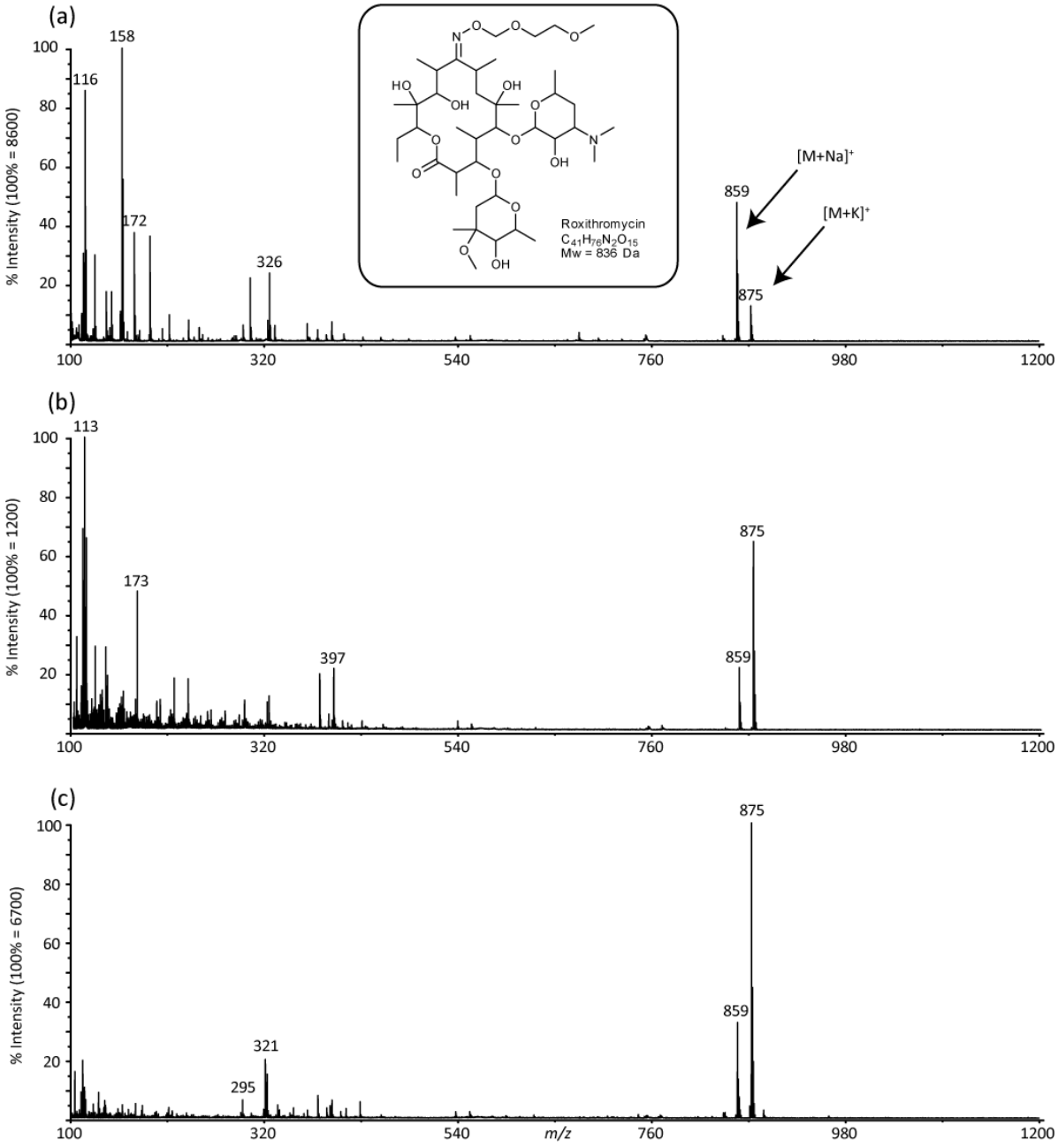
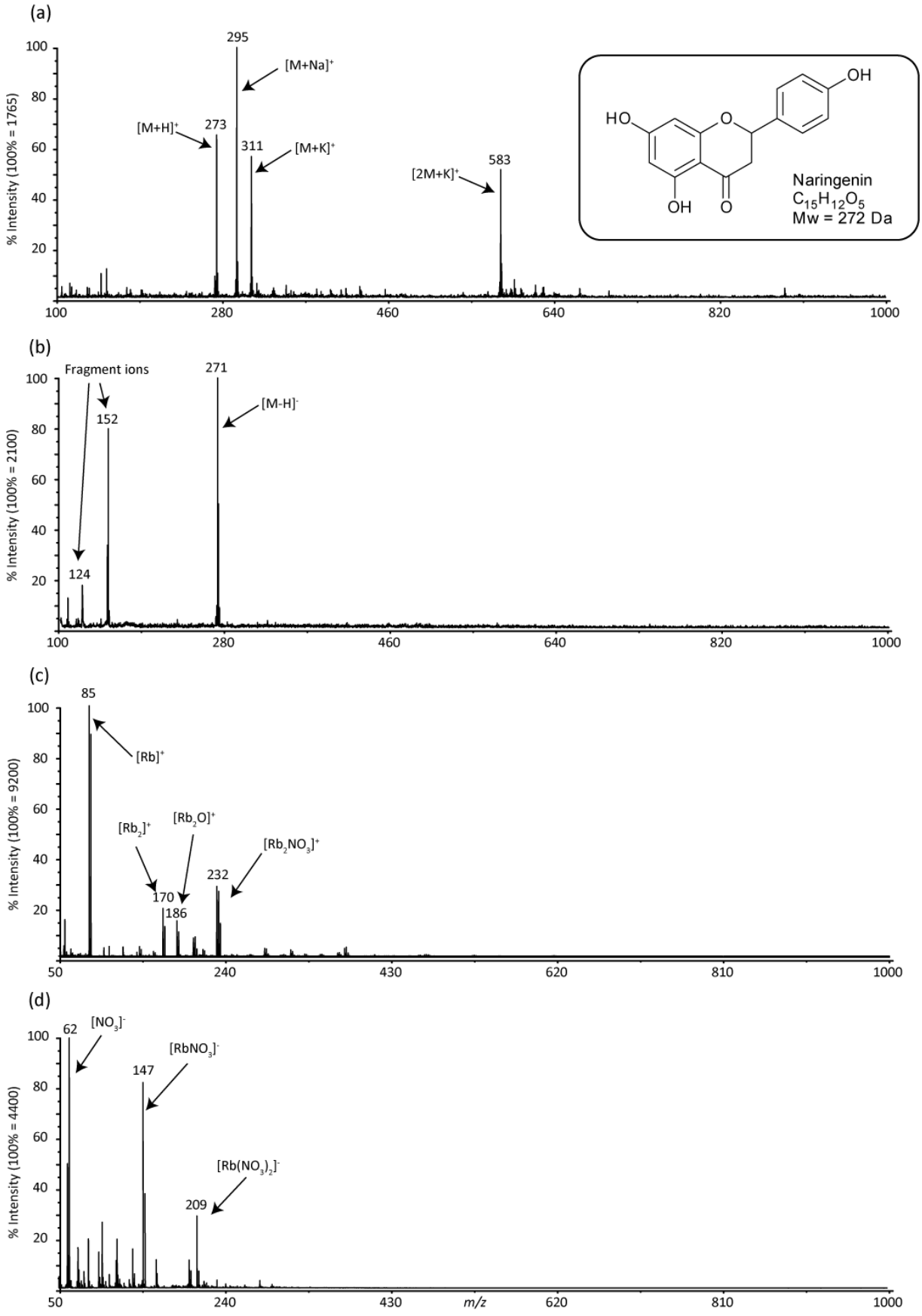

(a)
% Intensity (100% = 8600)
158
116
172
326
859
875
[M+Na]+
[M+K]+
Roxithromycin
C41H76N2O15
Mw = 836 Da
(b)
% Intensity (100% = 1200)
113
173
397
875
859
(c)
% Intensity (100% = 6700)
875
859
321
295
m/z
100
320
540
760
980
1200

Figure 4:


(a)
% Intensity (100% = 1765)
295
[M+Na]+
[M+H]+
273
311
[M+K]+
583
[2M+K]+
HO
OH
O
OH
O
Naringenin
C15H12O5
Mw = 272 Da
100
280
460
640
820
1000
(b)
% Intensity (100% = 2100)
Fragment ions
271
[M-H]-
152
124
(c)
% Intensity (100% = 9200)
85
[Rb]+
[Rb2]+
[Rb2O]+
[Rb2NO3]+
232
170
186
50
240
430
620
810
1000
(d)
% Intensity (100% = 4400)
62
[NO3]-
[RbNO3]-
147
[Rb(NO3)2]-
209
m/z